Reference: *Biol. Bull.* **175:** 122–131. (August, 1988)

Energy Metabolism During Anoxia and Recovery in Shell Adductor and Foot Muscle of the Gastropod Mollusc *Haliotis lamellosa:* Formation of the Novel Anaerobic End Product Tauropine

GERD GÄDE

Institut für Zoologie IV, Universität Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf 1, Federal Republic of Germany

Abstract. Metabolic responses to experimental anoxia and subsequent recovery, and to exercise were investigated in two different muscular tissues of the ormer, *Haliotis lamellosa.* The tissues are employed for different tasks by the animal. The foot is mainly responsible for slow gliding movements. The shell adductor muscle pulls down the shell for protection and in righting an animal which has been dislodged from the rocks. Tissue-specific differences in anaerobic energy metabolism occur. During 6 h of experimental anoxia, energy for both muscles was provided by arginine phosphate and co-fermentation of glycogen and aspartate. Glycolysis in the shell adductor muscle led mainly to the formation of the novel end product tauropine; D-lactate production predominated in the foot. This pattern is consistent with observed enzymatic profiles in the two muscles and with the equilibrium constants of the respective enzymes, tauropine and D-lactate dehydrogenase. Recovery from anaerobiosis was characterized by a rapid return of the phosphagen pool and the energy charge to the aerobic state. A protracted time-course was observed for the clearance of glycolytic end products.

Exercise, primarily powered by the shell adductor muscle, was mainly fueled by glycolysis resulting mostly in the accumulation of tauropine.

Introduction

In recent years different enzymes that terminate anaerobic glycolysis, so-called opine dehydrogenases, have been identified in the tissues of many marine invertebrates (for a review, see Gäde and Grieshaber, 1986). The products formed (octopine, strombine, or alanopine) via the reductive condensation of pyruvate with the respective amino acid (arginine, glycine or alanine) are collectively known as opines:

$$\text{pyruvate} + \begin{matrix}\text{arginine}\\ \text{glycine}\\ \text{alanine}\end{matrix} + \text{NADH} + \text{H}^+ \underset{\text{alanopine dehydrogenase}}{\overset{\text{octopine dehydrogenase}\\ \text{strombine dehydrogenase}}{\rightleftharpoons}} \begin{matrix}\text{octopine}\\ \text{strombine}\\ \text{alanopine}\end{matrix} + \text{NAD}^+ + \text{H}_2\text{O}.$$

Recently, a unique compound was detected in muscle extracts of the prosobranch gastropod, the abalone *Haliotis discus hannai* (Sato *et al.*, 1985). It was identified as D-rhodoic acid (now termed tauropine), previously isolated from some red algae (Kuriyama, 1961). The responsible enzyme, rhodoic acid dehydrogenase or tauropine dehydrogenase, catalyzing the reaction: pyruvate + taurine + NADH + $H^+ \rightleftharpoons$ tauropine + NAD^+ + H_2O, has been found in muscle tissue of the Japanese abalone as well as in the European ormer, *Haliotis lamellosa* (Sato and Gäde, 1986), and subsequently purified and characterized in detail in the latter species (Gäde, 1986). Certain features of this enzyme suggest a role in maintaining cytoplasmic redox balance during hypoxic conditions in the ormer (Gäde, 1986).

Various invertebrates can withstand hypoxic conditions in their habitats. Their metabolism during such a period of environmental anoxia is characterized by co-fermentation of aspartate and glycogen leading to the ac-

Received 15 March 1988; accepted 31 May 1988.

cumulation of the end products succinate, alanine and (in some cases) propionate and acetate. The rate of energy production is low, but the yield of ATP increased (reviewed by de Zwaan, 1977; Schöttler, 1980; Livingstone, 1982; Gäde, 1983a; Storey, 1985). During excessive locomotory activity, the capacities of muscle tissue to synthesize ATP rapidly by aerobic means are limited and energy provisions are met during functional anoxia via anaerobic glycolysis resulting in the accumulation of lactate or the opines. The rate of energy production is high, but the efficiency is low (references as above and Gäde, 1980; de Zwaan and van den Thillart, 1985; Gäde and Meinardus, 1986; Gäde and Grieshaber, 1986; Gäde, 1987a).

The present study concerns the anaerobic energy metabolism of muscle tissue in the ormer *Haliotis lamellosa*. The strategies used to provide energy during environmental and functional anoxia are of paramount importance for the survival of this species. The ormer is epifaunal in the littoral zone, attached by its foot to wave-swept rocks. The broad shell acts as a protective shield. It is pulled down tightly by the large shell adductor muscle (the right retractor or columella muscle) during low tide or vigorous wave action. When dislodged, ormers are extremely vulnerable to predators, especially since they often lie upside down. Therefore, these gastropods typically right themselves as fast as possible. This, again, is achieved mainly by relatively active movements of the large shell adductor muscle and with less input of the foot (unpub. obs.).

Previous studies revealed interesting patterns of dehydrogenase distribution in muscle tissue of the ormer (Gäde, 1986). Most tauropine dehydrogenase activity is found in the shell adductor muscle, which contains only minute levels of D-lactate dehydrogenase activity. In the foot muscle D-lactate dehydrogenase displays the highest activity. This almost mutually exclusive distribution of the dehydrogenases, combined with the different involvement of the muscle tissues during environmental (both tissues) and functional (mainly shell adductor) anoxia, led us to compare the energetics of the two muscle tissues. Furthermore, we investigated the metabolic events during recovery in well-aerated seawater, immediately following experimental anoxia, since data on the fate of the accumulated end products and re-charging of the depleted high energy phosphates are rather scarce (see review by Ellington, 1983).

This paper shows unequivocally that the fermentation of glycogen to the novel end product tauropine maintains cytoplasmic redox balance during experimental as well as functional anoxia in the shell adductor muscle. D-lactate is the main fermentation product in the foot during 6 h of anoxia. Both glycolytic end products are apparently oxidized very slowly *in situ*. Comparison of the glycolytic rates during experimental and functional anoxia reveals a 10-fold increase in the shell adductor, but only an enhancement by a factor of 2 in the foot muscle.

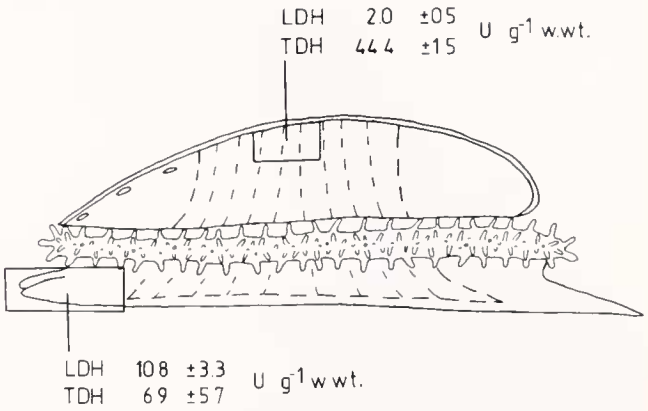

Figure 1. The sites of tissue-sampling (shell adductor muscle and foot) in the ormer, *Haliotis lamellosa*. The boxes identify the sites. Additionally, the enzyme activities for D-lactate dehydrogenase (LDH) and tauropine dehydrogenase (TDH) for each tissue are given as means ± 1 S.D. (n = 4) in units per gram wet weight (U g^{-1} wt wgt).

Materials and Methods

Animals and tissues

Specimens of the ormer *Haliotis lamellosa* (5–7 cm maximal shell length) were collected by local fishermen from the Bay of Naples, Italy, during October 1986. Animals were maintained in flowing seawater (22–24°C) at the Stazione Zoologica. Animals were used in experiments four to six days after collection.

Due to different profiles in the enzyme activities for pyruvate reductases (see Introduction), muscle tissue from two different organs were used in this study (Fig. 1): we compared the metabolic changes occurring in the shell adductor muscle to those in the foot. The dorsal part of the shell adductor (near the attachment of this muscle to the shell), and the anterior edge of the foot just below the head, were always excised for study (Fig. 1).

Biochemicals

Biochemicals were from Sigma Chemical Company (Deisenhofen, FRG) and Boehringer GmbH (Mannheim, FRG). All other chemicals were of reagent grade quality and came from Merck (Darmstadt, FRG). Tauropine dehydrogenase (EC 1.5.1.?), used to determine taurine and tauropine, was purified from the shell adductor muscle of *H. lamellosa* as outlined previously (Gäde, 1987b). D-lactate dehydrogenase (EC 1.1.1.28) and octopine dehydrogenase (EC 1.5.1.11), used to assay for D-lactate, arginine phosphate, and arginine, respectively,

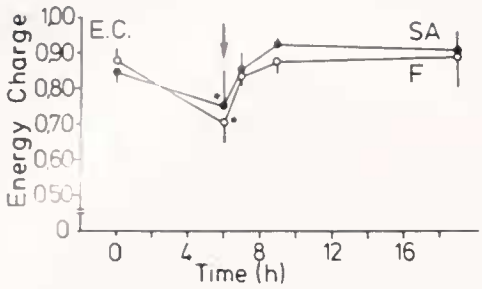

Figure 2. Alterations in the adenylate energy charge (E.C.) in the shell adductor (SA; solid circles) and foot (F; open circles) muscle of *Haliotis lamellosa* during environmental anoxia and recovery (onset marked by arrow). Each value is a mean ± 1 S.D. (for n, see Materials and Methods). The asterisks denotes a significant change compared to controls.

were purified from muscle tissue of the horseshoe crab, *Limulus polyphemus* (Carlsson and Gäde, 1985), and from the adductor muscles of the scallop, *Pecten jacobaeus* (Gäde and Carlsson, 1984), respectively.

Experimental procedure

Metabolic responses to environmental hypoxia and recovery. Twenty specimens of *H. lamellosa* were incubated in wash bottles (10 animals each) filled with about 2 l of seawater (22–24°C) that had been gassed with pure nitrogen until P_{O_2} (monitored with an oxygen electrode) reached almost zero mm Hg. After the animals were inserted, the wash bottles were flushed with a constant, slow stream of nitrogen gas. After 6 h of anoxic incubation, seven animals were removed, and their shell adductor and foot muscles excised, blotted, and frozen in liquid nitrogen. The remaining ormers were returned to well-aerated seawater, and subsets of four animals were removed at various intervals (1, 3, and 13 h) and treated as above. Furthermore, a zero time group of seven gastropods were chosen for the controls. The frozen tissues were subsequently transported from Naples to Düsseldorf on dry ice and stored at −35°C.

Metabolic responses to functional hypoxia. Four animals were exercised for 12 to 15 min in a large aquarium with flowing-seawater system. To induce exercise, the animals were placed upside-down on their shells; their righting movements involved, primarily, relatively vigorous contractions of the shell adductor muscle. When the animals had regained their normal posture, they were immediately inverted again. This work was continued for up to 15 min, when movements became much slower and the animals appeared to be exhausted. Shell adductor and foot muscles were then removed and treated as above.

Metabolite assays

Neutralized perchloric acid extracts were prepared from the frozen tissues of *H. lamellosa* according to previously published methods (Gäde *et al.*, 1978; Carlsson and Gäde, 1986). The levels of ATP, ADP, AMP, arginine, and arginine phosphate were determined spectrophotometrically by the methods of Lamprecht and Trautschold (1974), Jaworeck *et al.* (1974), and Gäde (1985a); the determinations were made immediately after neutralization of the extracts to eliminate sample losses.

Other metabolites were quantitated spectrophotometrically after storage of the extracts at −25°C. The methods used were those of Gawehn and Bergmeyer (1974) for D-lactate, Graβl (1974a, b) for L- and D-alanine, Bergmeyer *et al.* (1974) for aspartate, Williamson (1974) for succinate, and Gäde (1987b) for taurine. Tauropine was determined enzymatically using tauropine dehydrogenase in an assay system almost identical to that used for octopine quantification (Gäde, 1985b).

All metabolite data were analysed for significant

Table I

Alterations in the levels of adenylates (μmoles g^{-1} wt·wgt) in shell adductor and foot muscle of Haliotis lamellosa *during experimental anoxia and subsequent recovery*

Time (h) of anoxia or recovery	(n)	Shell adductor muscle				Foot muscle			
		ATP	ADP	AMP	Sum	ATP	ADP	AMP	Sum
Anoxia:									
0	7	3.25 ± 0.99*	0.96 ± 0.20	0.23 ± 0.16	4.44 ± 1.23	0.73 ± 0.31	0.14 ± 0.06	0.03 ± 0.01	0.90 ± 0.32
6	7	3.00 ± 0.68	1.43 ± 0.49	0.54 ± 0.36	4.97 ± 0.34	0.62 ± 0.32	0.51 ± 0.13	0.10 ± 0.04	1.23 ± 0.45
Recovery:									
1	4	3.04 ± 0.54	0.85 ± 0.17	0.19 ± 0.15	4.08 ± 0.64	0.41 ± 0.20	0.13 ± 0.08	0.02 ± 0.01	0.56 ± 0.28
3	4	3.81 ± 0.35	0.64 ± 0.08	0.05 ± 0.03	4.50 ± 0.36	0.70 ± 0.19	0.19 ± 0.08	0.03 ± 0.01	0.42 ± 0.25
13	4	3.09 ± 0.66	0.58 ± 0.21	0.09 ± 0.06	3.76 ± 0.50	0.91 ± 0.25	0.19 ± 0.11	0.04 ± 0.01	1.14 ± 0.38

* All values are given as mean ± SD.

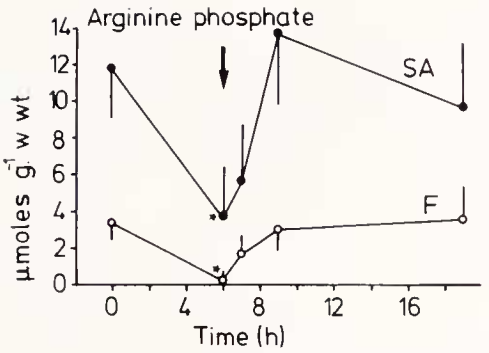

Figure 3. Time-course of the levels of arginine phosphate in the shell adductor (SA; solid circles) and foot (F; open circles) muscle of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

changes (anaerobic *versus* controls) by analysis of variance (ANOVAR) using confidence limits of $P \leqq 0.05$.

Results

Metabolic responses to environmental hypoxia and recovery

The levels of the adenylates in the shell adductor muscle and foot during 6 h anoxia and recovery are listed in Table I. The calculated adenylate energy charge (E.C. = ATP + ½ ADP ÷ ATP + ADP + AMP) is depicted in Figure 2. In control animals the adenylate content of the shell adductor muscle was more than 4-fold compared with the foot muscle. This was also true for the individual adenylates ATP, ADP, and AMP. The concentrations of ADP and AMP increased during anoxia, but, due to high variations, these changes were not statistically significant in either tissue, whereas the value of the energy charge declined significantly in both tissues. Upon recovery, ADP and AMP levels as well as the energy charge returned to near the control state after one h. For both tissues, 13 h of recovery after anoxia led to marginally higher values of the energy charge as estimated for control animals.

The sum of the arginine containing compounds (arginine and arginine phosphate) in control animals was about 4-fold higher in the shell adductor muscle (31 µmoles/g w. wt.) than in the foot muscle (7 µmoles/g w. wt.), and it stayed virtually constant throughout the experiment (results not shown). Arginine phosphate levels fell drastically in the shell adductor during anoxia. A significant drop was also seen in the foot (Fig. 3). Concomitantly, the arginine levels rose as a mirror image (results not shown). During recovery, arginine phosphate levels rose more slowly than the energy charge value in both tissues and reached initial levels after 3 h.

The aspartate levels in both tissues were the same in control ormers and there was a significant decline in both tissues upon anoxic incubation (Fig. 4). Whereas aspartate levels were not restored in the shell adductor during recovery, the levels increased slowly, but steadily, in the foot muscle. However, after 13 h of recovery, aspartate levels still differed from pre-anoxic exposure levels.

Levels of D- and L-alanine in control animals were 3- to 4-fold higher in the shell adductor than in the foot (Fig. 5). During anoxia, both stereoisomers accumulated significantly in the foot muscle; L-alanine accumulated in the shell adductor muscle. Alanine levels in the foot muscle returned to control levels during recovery (Fig. 5), but recovery was not evident in the shell adductor muscle.

Initial succinate levels in control animals were slightly higher in the shell adductor compared to the foot (Fig.

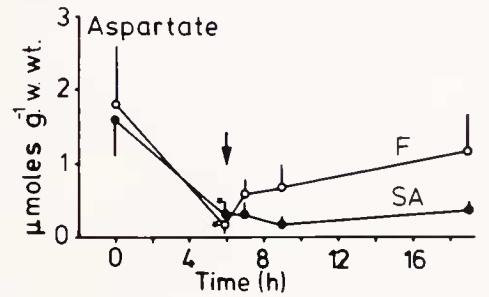

Figure 4. Time-course of the levels of aspartate in the shell adductor (SA; solid circles) and foot (F; open circles) muscle of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

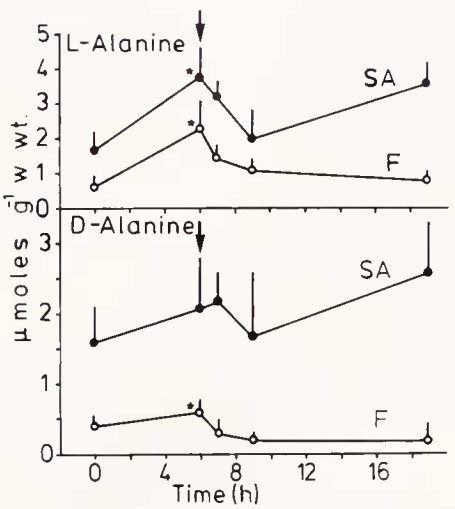

Figure 5. Time-course of the levels of L-alanine (upper panel) and D-alanine (lower panel) in the shell adductor (SA; solid circles) and foot (F; open circles) muscle of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

6). Anoxic incubation resulted in a significant increase in both tissues. In the foot muscle, initial values were rapidly achieved after 1 h of recovery. In contrast, succinate levels in the shell adductor declined more slowly, and took 3 h to reach pre-anoxic levels.

Taurine levels were about twice as high in the shell adductor as in the foot of control abalones. No significant changes occurred in the foot. There was a significant decline in the shell adductor muscle during anoxia without any restoration in the recovery period (Figs. 7, 8). Substantial accumulations of tauropine were evident in the shell adductor muscle during anoxia, while a small, but significant, formation occurred in the foot muscle. In the latter tissue, the main anaerobic end product was D-lactate, which also accumulated in the adductor, but to a much lesser extent (50%) than tauropine (Figs. 7, 8). D-lactate levels were rapidly cleared to 50% of the anoxic level during the first hour of recovery in the foot. Both tauropine and D-lactate levels remained high in the shell adductor after 3 h of recovery. Even after 13 h of recovery these levels were still higher than the initial concentrations before anoxia.

No significant changes in the levels of glucose-6-phosphate (0.50 and 0.15 μmoles/g w. wt. in shell adductor and foot muscle, respectively) were observed during anoxic incubation and recovery (results not shown).

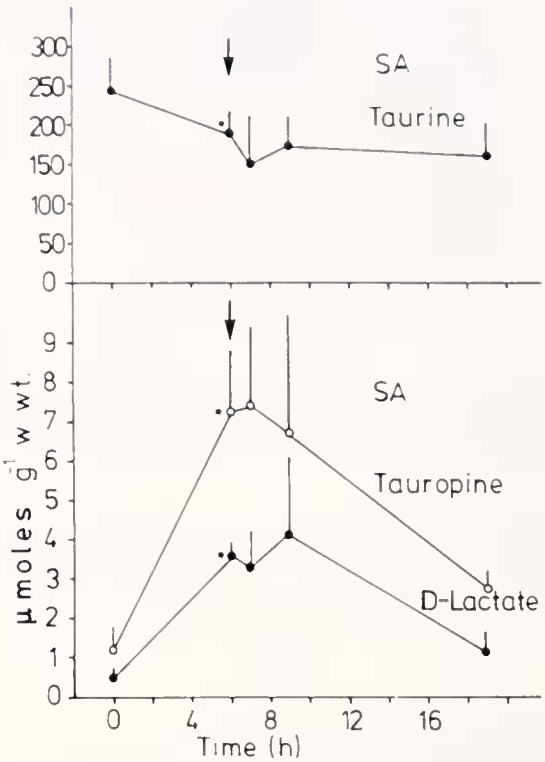

Figure 7. Time-course of the levels of taurine (upper panel) and (lower panel) D-lactate (solid circles) and tauropine (open circles) in the shell adductor muscle (SA) of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

Metabolic responses to functional hypoxia

The levels of the adenylates, arginine-containing compounds, and various other metabolites in the shell adductor muscle and the foot during exercise are listed in Table II. There was no significant change in the energy charge or in the levels of arginine phosphate in either tissue. Aspartate levels were marginally, but significantly, diminished in the shell adductor, whereas a small, but significant rise in the levels of L-alanine was observed in the foot. As main glycolytic end products, levels of D-lactate (marginally) and tauropine (primarily) were elevated significantly in the shell adductor muscle, whereas the D-lactate levels were doubled in the foot without any significant rise in the tauropine levels.

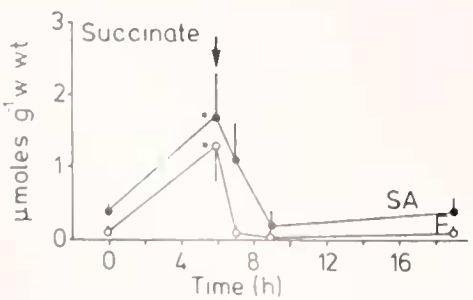

Figure 6. Time-course of the levels of succinate in the shell adductor (SA; solid circles) and foot (F; open circles) muscle of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

Discussion

This study in the ormer is a good example of the principle that anaerobic energy metabolism in a muscle is specifically matched to the function of the muscle. It reflects adaptation to specific metabolic need of the tissue. The two investigated tissues, the foot and the shell adductor muscle, are employed by the animal for different tasks. The foot is mainly responsible for slow gliding movements that very likely are supported by aerobic metabolism. In contrast, the shell adductor muscle pulls the ormer's shield-like shell tightly to the substratum to preclude dessication during low tide and to prevent dislodging by wave action. The shell adductor also rights an animal that has been detached from the rocks. Thus, the shell adductor muscle is metabolically more active than the foot and performs burst contractions which, in general, rely on anaerobic metabolism. However, when the whole animal has to cope with hypoxic or even anoxic conditions, both tissues need to have the capacity for maintaining metabolism anaerobically.

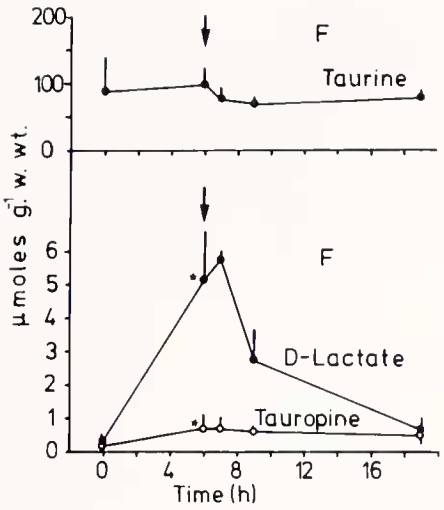

Figure 8. Time-course of the levels of taurine (upper panel) and (lower panel) D-lactate (solid circles) and tauropine (open circles) in the foot (F) of *Haliotis lamellosa* during experimental anoxia and recovery. For further details, see Figure 2.

This partitioning in function is also reflected in the metabolism of the two tissues. Compared to foot muscle, the shell adductor contains 4-fold higher levels of high-energy phosphates, ATP (and, in fact, the total adenylate pool), and arginine phosphate, suggesting a higher metabolic rate. This is confirmed when the energy demand for both tissues is calculated from the decreased levels of the phosphagens and the increased levels of glycolytic products occurring during experimental anoxia (Table III): the ATP production rate (μmoles g^{-1} wt wgt min^{-1}) for the shell adductor muscle is about twice as high as for the foot. In both tissues the bulk of the energy is provided by anaerobic glycolysis (between 70 and 80%), the remainder by the phosphagen (Table III). Again, the glycolytic flux (calculated in nmoles glycosyl units g^{-1} wt wgt min^{-1}; Table III) is also 1.5-fold higher in the shell adductor.

The main qualitative difference between the two tissues is the involvement of two different glycolytic end products in anaerobic metabolism. Whereas glycogen breakdown in foot results in the production of D-lactate, glycolysis in the shell adductor is terminated with the formation of the novel end product tauropine. Thus, the tauropine/tauropine dehydrogenase system—functionally analogous to the lactate/lactate dehydrogenase system—is active in the shell adductor muscle to maintain cytoplasmic redox balance. This pattern of end product formation in the different tissues is in agreement with the enzymatic complement of the respective tissue: lactate dehydrogenase is the predominant pyruvate reductase present in the foot; tauropine dehydrogenase is almost exclusively present in the shell adductor (Gäde, 1986).

We now ask why tauropine dehydrogenase turns up in this mollusc, and why taurine is used as a substrate for a dehydrogenase. Opine dehydrogenases which use the amino acids L-arginine, glycine, and L-alanine for the condensation reaction with pyruvate are already known (see review by Gäde and Grieshaber, 1986). Obviously, the opine dehydrogenases that have evolved are those that would make use of the most abundant amino acids in the species. The same is true for the amino acid taurine. From the amino acids used for opine production (arginine, glycine, alanine, taurine) it is the one with the highest concentration in the shell adductor muscle of the ormer (Gäde, 1986 and this study for arginine). There seems a sort of "co-evolution" of the most abundant amino acid and the corresponding opine dehydrogenase; thus, the specificity of the enzyme for the amino acid may be evolutionarily altered as a consequence of the "makeup" of the pool of amino acids in the different tissues. The mechanism of this is not understood yet, however another example is in the literature. In the polychaete worm *Aphrodite aculeata*, strombine dehydrogenase was found in pharynx muscle containing extremely high levels of glycine, whereas alanopine dehydrogenase was present in body wall musculature (Storey, 1983). Siegmund (1986; also cited in Grieshaber and Kreutzer, 1986) compared the concentrations of those amino acids involved in the action of octopin-, strombine-, and alanopine dehydrogenase from various marine invertebrates (coelenterates, molluscs, and annelids) to the amounts of octopine, strombine, and alanopine actually formed during environmental anoxia. He showed that in the species investigated the most abundant of these amino acids was used as a substrate for opine formation. This was the case for alanine/alanopine in *Littorina littoralis, L. littorea, Nucella lapillus,* and *Glycera convoluta,* and for glycine/strombine in *Halichondra panicea, Mytilus edulis, Crassostrea angulata, Pharus legumen, Solen marginatus, Ensis siliqua, Lutraria lutraria, Arenicola marina, Nephtys hombergi, Pherusa plumosa,* and *Pectinaria koreni.* However, tauropine production was not analyzed, but in many of the species taurine concentrations are higher than those of the other amino acids determined. Thus, with the present small data base available—tauropine dehydrogenase has additionally only reported from muscle tissue of the brachiopod *Glottidea pyramidata* (Doumen and Ellington, 1987)—it is not possible to conclude from a high taurine concentration to the presence of tauropine dehydrogenase and/or production of tauropine.

Another question concerning *H. lamellosa* is: what is the significance of tissue-specific differences in pyruvate metabolism? We speculate that the driving force that led to the appearance of tauropine dehydrogenase in the shell adductor muscle is the requirement for burst activ-

Table II

Levels of adenylates, various metabolites (μmoles g^{-1} wt·wgt) and adenylate energy charge in shell adductor and foot muscle of Haliotis lamellosa *during exercise (15 min)*

Metabolite	Shell adductor muscle		Foot muscle	
	Control (n = 7)	Exercise (n = 4)	Control (n = 7)	Exercise (n = 4)
ATP	3.25 ± 0.99*	2.65 ± 0.11	0.73 ± 0.31	1.00 ± 0.59
ADP	0.96 ± 0.20	1.02 ± 0.18	0.14 ± 0.06	0.20 ± 0.07
AMP	0.23 ± 0.16	0.14 ± 0.07	0.03 ± 0.01	0.05 ± 0.02
Sum	4.44 ± 1.23	3.81 ± 0.28	0.90 ± 0.32	1.25 ± 0.64
Energy charge	0.85 ± 0.04	0.83 ± 0.03	0.88 ± 0.03	0.86 ± 0.06
Arginine	19.63 ± 4.38	21.25 ± 4.00	3.56 ± 1.23	5.54 ± 2.46
Arginine phosphate	11.77 ± 2.92	9.88 ± 3.37	3.42 ± 1.18	4.41 ± 3.91
Sum	31.40 ± 2.31	31.13 ± 4.65	6.98 ± 2.27	9.95 ± 6.17
D-lactate	0.50 ± 0.10	0.86 ± 0.16**	0.33 ± 0.19	0.77 ± 0.15**
Tauropine	1.22 ± 0.57	5.83 ± 2.09**	0.23 ± 0.10	0.40 ± 0.26
Taurine	243.2 ± 39.50	168.90 ± 22.0**	91.6 ± 50.5	84.0 ± 23.9
L-alanine	1.72 ± 0.46	2.83 ± 1.48	0.56 ± 0.28	1.26 ± 0.57**
D-alanine	1.60 ± 0.45	1.09 ± 0.74	0.37 ± 0.18	0.18 ± 0.15
Aspartate	1.61 ± 0.47	0.60 ± 0.48**	1.77 ± 0.80	1.05 ± 0.66
Succinate	0.36 ± 0.14	0.40 ± 0.25	0.11 ± 0.03	0.10 ± 0.09

* All values given as mean ± SD.
** Significant to control.

ity creating functional anoxia during the righting movements. Such active muscle work needs a rapid activation of glycolytic energy production, which eventually leads to an increased redox status ($NADH/NAD^+$ ratio). Based on theoretical considerations of thermodynamic properties of opine and lactate dehydrogenases, it was proposed that the large amino acid pool used for opine production is decisive for maintaining the $NADH/NAD^+$ ratio lower than using the lactate pathway (see review by Fields, 1983). Most other arguments for the possible advantages of opine synthesis *versus* lactate formation (*e.g.*, lack of disturbance of internal osmolarity and less acidification) have been dismissed, as discussed recently by Grieshaber and Kreutzer (1986).

Besides glycogen and arginine phosphate breakdown, the amino acid aspartate provides energy during anoxia. The simultaneous depletion of aspartate and accumulation of succinate and alanine in both tissues indicates cofermentation of glycogen and aspartate as known to occur in other invertebrates during lack of oxygen (see, for example, Gäde, 1983a, 1987a; Kreutzer *et al.*, 1985). The observation of only very small amounts of succinate

Table III

Comparison of energy yield (μmoles ATP g^{-1} wt·wgt), rate of energy consumption (μmoles ATP g^{-1} wt·wgt min^{-1}), and glycolytic flux (nmoles glycosyl units g^{-1} wt·wgt min^{-1}) in shell adductor and foot muscle of Haliotis lamellosa *during experimental anoxia and exercise**

	ATP equivalents (μmoles g^{-1} wt wgt) from		Rate of consumption of ATP (μmoles g^{-1} wt·wgt min^{-1})	Glycolytic flux (nmoles glycosyl unit g^{-1} wt·wgt min^{-1})
	Glycolysis**	Phosphagen		
Six-hour experimental anoxia				
shell adductor	18.9 (70%)***	8.2	0.08	16.3
foot	12.2 (79%)	3.2	0.04	10.2
Exercise				
shell adductor	9.3 (81%)	2.2	0.77	206
foot	10 (100%)	—	0.10	22.7

* For calculations see Meinardus-Hager and Gäde, 1987.
** The small increase of succinate was assumed to derive by aspartate breakdown and is included in this calculation.
*** Contribution of glycolysis as percentage of total equivalents of ATP is given in brackets.

formed in both tissues of the ormer during anoxia and no production of propionate makes it highly unlikely that succinate is derived from glycogen by the so-called phosphoenolpyruvate carboxykinase route. This pathway is apparently only operative in "good anaerobes" tolerating prolonged hours of anoxia (see Introduction). The lack of propionate (and acetate) formation in the ormer is then indicative that this species can tolerate anoxic conditions for only a fraction of the time compared to species such as certain blue mussels, oysters, and many annelids. Indeed, preliminary experiments with specimens of *H. lamellosa* showed that these animals were unable to survive experimental anoxia longer than eight hours.

Recovery in both muscles of *H. lamellosa* was quite similar. Levels of the high-energy phosphates and succinate were rapidly restored, but asparate levels increased slowly (and only) in the foot. Similar changes have been reported during recovery in the foot of the cockles, *Cardium edule* (Gäde and Meinardus, 1981) and *C. tuberculatum* (Meinardus-Hager and Gäde, 1987), and the adductor muscle of the file shell, *Lima hians* (Gäde, 1983b).

Levels of the respective glycolytic end products in the tissues of the ormer, D-lactate and tauropine, were not cleared during the first h (foot) or 3 h (shell adductor) of recovery, but also did not significantly increase during this time period. Thus, it is highly unlikely that an anaerobic initial phase of recovery occurs as observed in tissues of the bivalves *Mytilus edulis* (de Zwaan *et al.*, 1983), *M. squamosus* (Nicchitta and Ellington, 1983), and *Crassostrea gigas* (Eberlee *et al.*, 1983).

The power output during exercise in the ormer is low relative to the jet propulsion of cephalopods and scallops or the jumps of the cockle, *C. tuberculatum*. The present study shows that energy demand increases for both muscles in comparison to experimental anoxia: about 10-fold for the shell adductor and about 2-fold in the foot (Table III). Since the energy is mainly or exclusively (foot) derived from glycolysis, the same increases are calculated for the glycolytic fluxes (Table III). These increases are small when compared to *C. tuberculatum*, where during jumping the glycolytic rate is enhanced 100-fold above the resting rate (Gäde and Meinardus-Hager, 1986). The calculations in Table III also show that the shell adductor muscle is particularly involved in exercise of the ormer; its rate of energy consumption and its glycolytic flux are about 8- to 9-fold higher than those of the foot. The bulk of the energy during exercise came from glycolytic tauropine formation in the shell adductor muscle, although there was also some D-lactate production. Thus, anaerobic breakdown of glycogen to tauropine also supports exercise metabolism, as it did metabolism during experimental anoxia.

The formation, during both experimental and functional anoxia, of both glycolytic end products—tauropine and D-lactate—in the shell adductor muscle may be explained by theoretical considerations of the equilibrium constants of the respective reactions. Since the degree of product formation is determined by the equilibrium constant (K_{eq}) of a reaction, we measured the K_{eq} constants for the reactions catalysed by tauropine (TDH) and D-lactate dehydrogenase (D-LDH). These were $K_{eq}(TDH) = ([pyruvate] \times [taurine] \times [NADH] \times [H^+]) \times ([tauropine] \times [NAD^+])^{-1} = 7.15 \times 10^{-13}$ *M* (Gäde, 1986) and $K_{eq}(D\text{-}LDH) = ([pyruvate] \times [NADH] \times [H^+]) \times ([D\text{-}lactate] \times [NAD^+])^{-1} = 1.3 \times 10^{-12}$ (Gäde and Meinardus-Hager, 1986).

We can use these values to assess whether this theoretical equilibrium is reached *in vivo* by both enzymes. The actual equilibrium is given by the mass action ratio, *e.g.*, $MAR_{LDH} = ([pyruvate] \times [NADH] \times [H^+]) \times ([D\text{-}lactate] \times [NAD^+])^{-1}$ and $MAR_{TDH} = ([pyruvate] \times [taurine] \times [NADH] \times [H^+]) \times ([tauropine] \times [NAD^+])^{-1}$.

These ratios, however, were not calculated because data for neither the internal proton concentration, nor the $NAD^+/NADH$ ratio, are available for the shell adductor muscle. However, assuming that the reaction catalysed by LDH is at or near equilibrium in most biological systems, we can obtain indirect information on the equilibrium situation of the TDH reaction using the K_{eq} values (see above). The ratio $K_{eq}(TDH)/K_{eq}(D\text{-}LDH)$ has the value of 0.55 *M* and corresponds to the quotient $([D\text{-}lactate] \times [taurine]) \times ([tauropine])^{-1}$, since TDH and LDH share common substrates such as pyruvate, NADH, H^+, and NAD^+ (see above). Thus, if we use the concentrations of D-lactate, taurine, and tauropine measured in the shell adductor, the calculated ratio should be close to the theoretical value, 550 m*M*, if *both* reactions are near equilibrium (our assumption).

Table IV shows that the reactions are not exactly at the theoretical equilibrium, but the "close equilibrium" of neither reaction changed much during anoxia and subsequent recovery. Thus, the formation of primarily tauropine and a little D-lactate is according to the equilibrium constants of the reactions. In contrast, after exercise the quotient was 10-fold lower than the theoretical value indicating that the reactions of D-LDH and/or TDH are in "disequilibrium." Our data do not indicate which reaction that is, but a recent paper on a similar phenomenon in foot muscle of *C. tuberculatum* argues for a "disequilibrium" of D-LDH because of its low activity and the enhanced production of pyruvate and NADH due to the increased glycolytic flux (see details in Gäde and Meinardus-Hager, 1986; pages 197 and 198). The same arguments can be applied to the ormer during exercise: D-LDH activity in the shell adductor is much lower than TDH activity and glycolytic flux is enhanced.

Table IV

Calculation of the quotient $\frac{[taurine]}{[tauropine]}$ *for shell adductor muscle of* Haliotis lamellosa *during experimental anoxia, recovery, and exercise**

	Control	6-h anoxia	1-h recovery	3-h recovery	13-h recovery	Exercise
Quotient (m*M*	199	190	133	217	133	50

* Tissue concentrations calculated from data presented in Table II and Figures 7 and 8 under the assumption that the water content of the tissues is 50%.

In conclusion, the energy metabolism in both the shell adductor and foot muscle is powered by co-fermentation of glycogen and aspartate and transphosphorylation during experimental anoxia. According to differences in tissue activities, as well as thermodynamic properties of the two pyruvate reductases (TDH, LDH), tauropine is the preferred end product in the shell adductor, while D-lactate accumulation occurs in the foot. Recovery in well-aerated seawater reverses most of the metabolic changes seen during anoxia, but the time courses for high-energy phosphates (fast) and glycolytic end products (slow) are quite different, as in other molluscs. Enhanced glycolysis and maintenance of redox balance by the reaction of tauropine dehydrogenase are the main features of exercise in the shell adductor, and the foot is only minimally involved during this behavior.

Acknowledgments

The author thanks Dr. Georg Meinardus-Hager for help with the experiments, Dr. W. Ross Ellington (The Florida State University, Tallahassee) for commenting on and correcting the manuscript, and the staff of the Stazione Zoologica di Napoli for their hospitality. Financial support was provided in part by grants from the Deutsche Forschungsgemeinschaft (Ga 241/4-3 and Gr 456/12-1) and by a Heisenberg Fellowship awarded from the Deutsche Forschungsgemeinschaft (Ga 241/5-2).

Literature Cited

Bergmeyer, H. U., E. Bernt, H. Möllering, and G. Pfleiderer. 1974. L-Aspartat und L-Asparagin. Pp. 1741–1745 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Carlsson, K.-H., and G. Gäde. 1985. Isolation and characterisation of tissue-specific isoenzymes of D-lactate dehydrogenase from muscle and hepatopancreas of *Limulus polyphemus*. *J. Comp. Physiol.* **B155:** 723–731.

Carlsson, K.-H., and G. Gäde. 1986. Metabolic adaptation of the horseshoe crab, *Limulus polyphemus*, during exercise and environmental hypoxia and subsequent recovery. *Biol. Bull.* **171:** 217–235.

Doumen, C., and W. R. Ellington. 1987. Isolation and characterization of a taurine-specific opine dehydrogenase from the pedicles of the brachiopod, *Glottidea pyramidata*. *J. Exp. Zool.* **243:** 25–31.

Eberlee, J. C., J. M. Storey, and K. B. Storey. 1983. Anaerobiosis, recovery from anoxia and the role of strombine and alanopine in the oyster, *Crassostrea virginica*. *Can. J. Zool.* **61:** 2682–2689.

Ellington, W. R. 1983. The recovery from anaerobic energy metabolism in invertebrates. *J. Exp. Zool.* **228:** 431–444.

Fields, J. H. A. 1983. Alternatives to lactic acid: possible advantages. *J. Exp. Zool.* **228:** 445–457.

Gäde, G. 1980. Biological role of octopine formation in marine molluscs. *Mar. Biol. Lett.* **1:** 121–135.

Gäde, G. 1983a. Energy metabolism of arthropods and mollusks during environmental and functional anaerobiosis. *J. Exp. Zool.* **228:** 415–429.

Gäde, G. 1983b. Energy production during anoxia and recovery in the adductor muscle of the file shell, *Lima hians*. *Comp. Biochem. Physiol.* **76B:** 73–77.

Gäde, G. 1985a. Arginine and arginine phosphate. Pp. 424–431 in *Methods of Enzymatic Analysis,* Vol. VIII, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Gäde, G. 1985b. Octopine. Pp. 419–423 in *Methods of Enzymatic Analysis,* Vol. VIII, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Gäde, G. 1986. Purification and properties of tauropine dehydrogenase from the shell adductor muscle of the ormer, *Haliotis lamellosa*. *Eur. J. Biochem.* **160:** 311–318.

Gäde, G. 1987a. Leben ohne Sauerstoff: Die Rolle der anaeroben Glykolyse bei aquatischen wirbellosen Tieren. *Verh. Dtsch. Zool. Ges.* **80:** 93–110.

Gäde, G. 1987b. A specific enzymatic method for the determination of taurine. *Biol. Chem. Hoppe-Seyler* **368:** 1519–1523.

Gäde, G., and K.-H., Carlsson. 1984. Purification and characterisation of octopine dehydrogenase from the marine nemertean *Cerebratulus lacteus* (Anopla: Heteronemertea): comparison with scallop octopine dehydrogenase. *Mar. Biol.* **79:** 39–45.

Gäde, G., and M. K. Grieshaber. 1986. Pyruvate reductases catalyze the formation of lactate and opines in anaerobic invertebrates. *Comp. Biochem. Physiol.* **83B:** 255–272.

Gäde, G., and G. Meinardus. 1981. Anaerobic metabolism in the common cockle *Cardium edule*. V. Changes in the levels of metabolites in the foot during aerobic recovery after anoxia. *Mar. Biol.* **65:** 113–116.

Gäde, G., and G. Meinardus-Hager. 1986. Anaerobic energy metabolism in Crustacea, Xiphosura and Mollusca: lactate fermentation versus multiple fermentation products. *Zool. Beitr. N. F.* **30:** 187–203.

Gäde, G., E. Weeda, and P. A. Gabbott. 1978. Changes in the level of octopine during the escape responses of the scallop *Pecten maximus* L. *J. Comp. Physiol.* **124:** 121–127.

Gawehn, K., and H. U. Bergmeyer. 1974. D-Laktat. Pp. 1538–1541 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Graßl, M. 1974a. L-Alanin. Pp. 1727–1730 in *Methoden der Enzy-*

matischen Analyse, Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Graßl, M. 1974b. D-Alanin. Pp. 1731–1734 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Grieshaber, M. K., and U. Kreutzer. 1986. Opine formation in marine invertebrates. *Zool. Beitr. N. F.* **30:** 205–229.

Jaworeck, D., W. Gruber, and H. U. Bergmeyer. 1974. Adenosin-5′-diphosphat und Adenosin-5′-monophosphat. Pp. 2179–2181 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Kreutzer, U., B. Siegmund, and M. K. Grieshaber. 1985. Role of coupled substrates and alternative end products during hypoxia tolerance in marine invertebrates. *Mol. Physiol.* **8:** 371–392.

Kuriyama, M. 1961. Ninhydrin reactive substances in marine algae—III. On the chemical structure of "unknown A" isolated from red algae. *Bull. Jpn. Soc. Sci. Fish.* **27:** 699–702.

Lamprecht, W., and I. Trautschold. 1974. ATP. Bestimmung mit Hexokinase und Glucose-6-phosphat-Dehydrogenase. Pp. 2151–2159 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

Livingstone, D. R. 1982. Energy production in the muscle tissue of different kinds of molluscs. Pp. 257–274 in *Exogenous and Endogenous Influences on Metabolic and Neural Control,* Vol. 1, A. D. F. Addink and N. Spronk, eds. Pergamon Press, Oxford.

Meinardus-Hager, G., and G. Gäde. 1987. Recovery from environmental anaerobiosis and muscular work in the cockle, *Cardium tuberculatum:* oxygen debt and metabolic responses. *J. Exp. Zool.* **242:** 291–301.

Nicchitta, C. V., and W. R. Ellington. 1983. Energy metabolism during air exposure and recovery in the high intertidal bivalve mollusc *Geukensia demissa granosissima* and the subtidal bivalve molluscs *Modiolus squamosus. Biol. Bull.* **165:** 708–722.

Sato, M., and G. Gäde. 1986. Rhodoic acid dehydrogenase: a novel amino acid-linked dehydrogenase from muscle tissue of *Haliotis* species. *Naturwissenschaften* **73:** 207–209.

Sato, M., N. Kanno, and Y. Sato. 1985. Isolation of D-rhodoic acid from the abalone muscle. *Bull. Jpn. Soc. Sci. Fish.* **51:** 1681–1683.

Schöttler, U. 1980. Der Energiestoffwechsel bei biotopbedingter Anaerobiose: Untersuchungen an Anneliden. *Verh. Dtsch. Zool. Ges.* **73:** 228–240.

Siegmund, B. 1986. Funktionsbedingte und biotopbedingte Hypoxien: Zur Bedeutung der Opine im anaeroben Energiestoffwechsel. Ph. D. Thesis, Universität Düsseldorf.

Storey, K. B. 1983. Tissue-specific alanopine dehydrogenase and strombine dehydrogenase from the sea mouse, *Aphrodite aculeata* (Polychaeta). *J. Exp. Zool.* **225:** 369–378.

Storey, K. B. 1985. A re-evaluation of the Pasteur effect: New mechanisms in anaerobic metabolism. *Mol. Physiol.* **8:** 439–461.

Williamson, J. R. 1974. Succinat. Pp. 1661–1666 in *Methoden der Enzymatischen Analyse,* Vol. 2, H. U. Bergmeyer, ed. Verlag Chemie, Weinheim.

de Zwaan, A. 1977. Anaerobic energy metabolism in bivalve molluscs. *Oceanogr. Mar. Biol.* **15:** 103–187.

de Zwaan, A., and G. van den Thillart. 1985. Low and high power output modes of anaerobic metabolism: Invertebrate and vertebrate strategies. In *Circulation, Respiration, and Metabolism,* R. Gilles, ed. Springer-Verlag, Berlin, Heidelberg.

de Zwaan, A., A. M. T. de Bont, W. Zurburg, B. L. Bayne, and D. R. Livingstone. 1983. On the role of strombine formation in the energy metabolism of adductor muscle of a sessile bivalve. *J. Comp. Physiol.* **149:** 557–563.